EXPERIMENTAL STUDIES OF FAT METABOLISM WITH DETERMINATIONS OF RESPIRATORY QUOTIENT AND KETONE BODY PRODUCTION OF TISSUES

AUTHOR(S):

SHIGENAGA, MASAYUKI

EXPERIMENTAL STUDIES ON FAT METABOLISM WITH DETERMINATIONS OF RESPIRATORY QUOTIENT AND KETONE BODY PRODUCTION OF TISSUES

by

MASAYUKI SHIGENAGA

From the 2nd Surgical Division, Kyoto University Medical School
(Director: Prof. Dr. YASUMASA AOYAGI)

Received for publication Sept. 20. 1957

INTRODUCTION

Rapid progress in understanding fat metabolism in vivo has been made since QUASTEL (1935) used tissue slices, and LELOIR-MUNOZ (1939) utilized washed liver particles in the study of the oxidation of fatty acids. The sequence of fat metabolism which is generally recognized at present is as follows: fatty acid breaks down to acetyl coenzyme A in the fatty acid cycle (after Lynen) and, in the presence of oxaloacetic acid, enters into the Krebs' Tricarboxylic Acid cycle (T.C.A. cycle) and is oxidized to water and carbon dioxide.

Independent of Geyer, Shafiroff and other studies, in 1949, HIKASA in our laboratory succeeded in producing a fat emulsion which could be safely given intravenously. Since then our histochemical and biochemical studies on the process of fat metabolism in vivo have been continued. NISHINO in our laboratory reported that the oxygen consumption of various tissues was markedly increased by the intravenous administration of the fat emulsion. While these findings show that the infused fat is well oxidized in the body, the pathway of utilization of fat introduced by the intravenous route is not conclusively known.

Therefore, the author attempted to elucidate this problem by studying both the respiratory quotient (R.Q.) and ketone body formation of rat tissues following intravenous administration of fat emulsion. A.R.Q. is defined as the ratio CO_2 produced to O_2 consumed, and this value serves to indicate only to a certain extent the nature of the metabolic process being studied. For instance, a R.Q. of 1.0 would occur upon complete oxidation of carbohydrate, 0.8 for protein and 0.7 for fat.

The significance of the R.Q. values was greatly clarified by the determination of the ketone bodies which were produced in the same tissues.

MATERIALS AND METHODS

A) *EXPERIMENTAL MATERIALS*

1) Fat Emulsion: In the present investigation, 20 per cent sesame oil emulsion and 20 per cent cod liver oil emulsion, both containing 7 per cent glucose and small quantities of stabilizers, were employed. The standard dose of the intravenous administration of the fat emulsion was defined as 1.5cc of 20 per cent fat emulsion,

which is equal to 0.3g fat, per 100g body weight.

2) Experimental Animals: Healthy male rats representing omnivorous animals, each weighing approximately 150g, were used. They had been maintained on fixed diet for 5 to 7 days and were fasted for 12 hours prior to the experiment, and were in the postabsorptive state.

3) Preparation of Tissue Slices: After injection of the fat emulsion, animals were successively sacrificed by bleeding without anesthesia at definite intervals.

The organs were excised and immediately lined with filter paper dampened with the buffer solution. Slices approximately 0.3mm thick were cut free hand with a safety razor blade.

4) Warburg's Apparatus: The instrument, which was improved by Nishino, was used and the temperature of water bath was kept to $37.5 \pm 0.05°C$.

5) Used Drugs: Methinonine as l-methionine, Vitamin C as l-ascorbic acid and pantothenic acid as calcium pantothenate were used in the formation of the solution.

B) *EXPERIMENTAL METHODS*

1) Procedure for the Determination of R.Q.: The measurement of R.Q. of tissue was made by the modified Dickens-Simer's lst method.

Three flasks, having one main chamber and two sidearms, and a standard thermobalometer were prepared. Each main chamber was filled with 2cc of saline-phosphate buffer solution containing 0.2 per cent glucose (8.5cc of m/2 Na_2HPO_4 solution, 1.5cc of m/2 KH_2PO_4 solution, 240cc of 0.9 per cent NaCl solution and 10cc of 5 per cent glucose solution) as the medium for the tissue slices, which were placed in flask 2 and 3. Alkali (0.4cc of 10 per cent KOH) and acid (0.4cc of 3n H_2SO_4) were placed respectively in the two sidearms of each flask and the gaseous phase was filled with 100 per cent oxygen.

At first, the acid and alkali were tipped from the sidearms into the main chamber to obtain the initial bound CO_2 in flask 1 (X_1CO_2) and flask 2 (X_2CO_2). After the oxygen uptake for 1 hour was determined in flask 3 (XO_2), the solutions were mixed to obtain the total CO_2 containing the bound CO_2 and the CO_2 evolved over the entire experimental period (X_3CO_2).

Hence, the R.Q. can be calculated by the following formula.

$$\text{R.Q.} = \frac{X_3CO_2 - (X_2CO_2 - X_1CO_2)\dfrac{m_2}{m_1} - X_1CO_2}{XO_2}$$

m_1: dry weight of tissue slices in flask 2.

m_2: dry weight of tissue slices in flask 3.

2) Procedure for the Determination of Ketone Body Production.

The determination of ketone body which was produced in tissue was performed by aniline-citrate method using Warburg's apparatus and total ketone body was expressed as acetoacetic acid. The main chamber of the flask was filled with 0.7cc of 50 per cent citric acid, 0.2cc of destilled water and the sidearm was filled with 0.9cc of the mixed solution in flask 3 following the determination of the R.Q. A control experiment was made by using the mixed solution in the above mentioned flask 1 in the place of the mixed solution in flask 3.

RESULTS AND DISCUSSION

A) *CHANGES IN THE R. Q. OF VARIOUS TISSUES FOLLOWING THE INTRAVENOUS ADMINISTRATION OF THE FAT EMULSION.*

1) Infusion of Sesame Oil Emulsion Alone (Group A).

Healthy male rats in the postabsorptive state were killed by bleeding without anesthesia at definite intervals after infusion of the standard dose of the sesame oil emulsion and Qo_2, Qco_2 and R. Q. of liver, kidney, spleen, lung, cardiac muscle and skeletal muscle were measured. The results are shown in Table 1. The oxygen

Table 1 Qo_2, Qco_2 and R.Q. of Various Tissues Following Intravenous Infusion of Sesame Oil Emulsion into Rats. (Mean)

Time after infusion		0	1 hr.	3 hrs.	6 hrs.	9 hrs.	12 hrs.
Liver	Qo_2	7.30	9.56	13.94	16.94	17.73	18.68
	Qco_2	6.35	7.55	8.92	8.34	10.64	13.26
	R.Q.	0.87	0.79	0.64	0.51	0.60	0.71
Kidney	Qo_2	21.09	22.89	27.93	32.14	33.05	34.09
	Qco_2	18.35	19.00	20.95	22.50	24.46	26.59
	R.Q.	0.87	0.83	0.75	0.70	0.74	0.78
Spleen	Qo_2	10.15	11.24	14.06	16.60	17.27	18.25
	Qco_2	8.83	9.44	10.69	12.28	13.13	14.24
	R.Q.	0.87	0.85	0.76	0.74	0.76	0.78
Lung	Qo_2	10.51	11.84	12.80	13.49	15.28	16.80
	Qco_2	9.25	9.95	10.11	10.12	11.77	13.27
	R.Q.	0.88	0.84	0.79	0.75	0.77	0.79
Cardiac muscle	Qo_2	5.42	5.82	6.39	6.72	7.40	7.70
	Qco_2	4.72	4.83	5.05	5.04	5.62	6.08
	R.Q.	0.87	0.83	0.79	0.75	0.76	0.79
Skeletal muscle	Qo_2	1.30	1.36	1.43	1.52	1.63	1.66
	Qco_2	1.14	1.16	1.14	1.16	1.26	1.33
	R.Q.	0.88	0.85	0.80	0.76	0.77	0.80

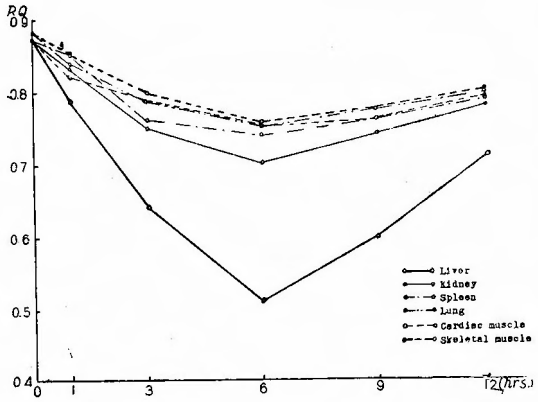
consumption of each tissue was increased gradually, particularly in liver, by the intravenous injection of the sesame oil emulsion. This finding, similar to the results obtained by Nishino, indicates that the infused fats are well oxidized in these tissues.

On the other hand, the R. Q. of each tissue began to decrease 1 hour after infusion and reached its minimum titer after 6 hours. Thereafter the titers decreased and were slightly lower than the pre-experimental titer 12 hours after infusion. The decrease in the R.Q. of liver was far more remarkable than that of the extrahepatic tissues as shown in Fig. 1. Thus, in our determination an obvious difference in R.Q. between liver and the extrahepatic tissues was recognized.

A decrease in the value of R.Q. is generally observed in the case of relative increase in O_2-uptake to CO_2-evolution, such as in the case of complete oxidation of substances containing fewer carbon atoms compared with oxygen atoms, or in the

case of the production of any metabolic intermediate. The R.Q. in complete oxidation of fat shows approximately 0.7. Why did the value of R. Q. in liver 6 hours after infusion of sesame oil emulsion decrease to around 0.5?

Fig. 1 Changes in R.Q. of Various tissues Following Intravenous Infusion of Sesame Oil Emulsion into Rats.



Asada and Izukura in our laboratory had carried out histochemical studies on the metabolic processes of fat with fat emulsions, and found that the intravenously infused fat globules were first phagocytized by the reticuloendothelial cells in the lung, liver and spleen, changing gradually into phospholipides in these cells, and then appeared in the hepatic parenchymatous cells in large quantities. Furthermore, Hashino in our laboratory demonstrated that ketone body levels in the blood and in the circulating fluid of isolated liver increased after infusion of fat emulsions. These findings and the results of the present experiments indicate that a part of infused fat converts to ketone body in the liver, even if another part of the fat is oxidized directly to water and carbon dioxide in this tissue.

However, in this investigation, the R.Q. in the extrahepatic tissues decreased 6 hours after infusion of the sesame oil emulsion to around 0.7. Accordingly, we can not help considering that the intravenously infused fat is not only oxidized finally in the extrahepatic tissues after conversion to ketone body, which is produced in the liver, but also oxidized directly in the same tissues from the form of phospholipide to water and carbon dioxide.

2) Simultaneous Infusion of Methionine with Sesame Oil Emulsion (Group B).

Recently, Artom and Entenman have emphasized that choline, which is synthetized by methionine, promotes fatty acid oxidation in the liver. Asada, Izukura and Hashino in our laboratory demonstrated that methionine accelerated phagocytosis and the conversion into phospholipide of fat by the reticuloendothelial cells, and secondarily expedited fatty acid oxidation, at least to the stage of ketone body, in the hepatic parenchymatous cells.

These effects of methionine were reexamined by manometric determination of tissue metabolism. The rats were injected intravenously the above mentioned standard dose of the sesame oil emulsion with 3mg of l-methionine per 100g body weight, and sacrificed at definite intervals after infusion for the measurement of Qo_2, Qco_2 and R.Q. of various tissues. The results are shown in Table 2. In this group, the rate of increase in Qo_2 of liver was higher than that in the case of the infusion of the sesame oil emulsion alone. On the other hand, the lowest value of the R.Q. of liver 6 hours after infusion presented almost no significant difference from the group A, though the values of 1 and 3 hour cases were slightly lower, and the values of

Table 2 Qo_2, Qco_2 and R.Q. of Various Tissues Following Simultaneous Infusion of Methionine with Sesame Oil Emulsion into Rats. (Mean)

Time after infusion		0	1 hr.	3 hrs.	6 hrs.	9 hrs.	12 hrs.
Liver	Qo_2	7.30	10.12	14.45	18.16	19.23	20.01
	Qco_2	6.35	7.59	8.53	9.62	12.31	14.41
	R.Q.	0.87	0.75	0.59	0.53	0.64	0.72
Kidney	Qo_2	21.09	23.90	29.60	33.24	34.56	35.14
	Qco_2	18.35	19.60	21.90	23.27	26.26	28.21
	R.Q.	0.87	0.82	0.74	0.70	0.76	0.80
Spleen	Qo_2	10.15	11.20	15.30	17.28	17.48	18.36
	Qco_2	8.83	9.30	11.78	13.13	13.46	14.83
	R.Q.	0.87	0.83	0.77	0.76	0.77	0.81
Lung	Qo_2	10.51	11.84	13.37	14.61	15.98	16.61
	Qco_2	9.25	9.95	10.56	11.25	12.30	13.28
	R.Q.	0.88	0.84	0.79	0.77	0.77	0.80
Cardiac muscle	Qo_2	5.42	5.92	6.48	6.89	7.49	7.71
	Qco_2	4.72	4.91	5.05	5.17	5.82	6.25
	R.Q.	0.87	0.83	0.78	0.75	0.78	0.81
Skeletal muscle	Qo_2	1.30	1.42	1.56	1.62	1.64	1.70
	Qco_2	1.14	1.18	1.22	1.20	1.26	1.38
	R.Q.	0.88	0.83	0.78	0.74	0.77	0.81

9 and 12 hour cases were slightly higher than those of its group. Thus, it was observed that methionine speeded up lightly the decreasing of the R.Q. of liver.

There was no evidence that the R.Q. of kidney, spleen, lung, cardiac muscle and skeletal muscle was decreased by methionine, although the oxygen consumption of these tissues in Group B increased much significantly than that in Group A. These results show that methionine accelerates the formation of ketone body in liver after infusion of the sesame oil emulsion.

3) Simultaneous Infusion of Methionine, F. A. D., Vitamin C and Pantothenic Acid with Sesame Oil Emulsion (Group C).

According to the experimental results, obtained by Hashino, the utilization of ketone body in liver is augmented by the addition of various vitamins, such as riboflavin, ascorbic acid and pantothenic acid, which are concerned in fat metabolism. Therefore the author carried out the following experiment to clarify the effects of these drugs. Methionine, 3mg, F.A.D. (Flavin Adenine Dinucleotide), 3mg, ascorbic acid, 6mg, and pantothenic acid, 6mg per 100g body weight were injected intravenously into rats with the standard dose of the sesame oil emulsion.

The results are presented in Table 3. In comparison to the previous groups, the great increase in oxygen consumption of all tissues was found in this group. Further, the decrease in the R.Q. of liver tended to be less than that of Group A and Group B and the R.Q. of the extrahepatic tissues, especially of spleen, lung and cardiac muscle, decreased more distinctly than that of those groups.

Table 3 Qo_2, Qco_2 and R.Q. of Various Tissues Following Simultaneous Infusion of Methionine, F.A.D., Vitamin C and Pantothenic Acid with Sesame Oil Emulsion into Rats. (Mean)

Time after infusion		0	1 hr.	3 hrs.	6 hrs.	9 hrs.	12 hrs.
Liver	Qo_2	7.30	10.22	14.67	18.47	20.07	20.73
	Qco_2	6.35	7.46	9.53	11.08	12.44	14.51
	R.Q.	0.87	0.73	0.65	0.60	0.62	0.70
Kidney	Qo_2	21.09	24.96	31.97	35.04	35.23	36.00
	Qco_2	18.35	19.97	23.36	24.87	26.06	27.77
	R.Q.	0.87	0 80	0.73	0.71	0.74	0.77
Spleen	Qo_2	10.15	11.38	16.20	18.36	19.01	19.78
	Qco_2	8.83	8.99	12.06	13.18	14.05	15.00
	R.Q.	0.87	0.80	0.74	0.72	0.74	0.76
Lung	Qo_2	10.51	12.09	13.68	15.07	17.06	18.00
	Qco_2	9.25	9.80	10.16	10.91	12.72	13.82
	R.Q.	0.88	0.81	0.74	0.71	0.75	0.77
Cardiac muscle	Qo_2	5.42	6.06	6.96	7.20	7.68	8.06
	Qco_2	4.72	4.97	5.26	5.17	5.75	6.21
	R.Q.	0.87	0.82	0.76	0.72	0.75	0.77
Skeletal muscle	Qo_2	1.30	1.46	1.64	1.69	1.74	1.83
	Qco_2	1.14	1.20	1.26	1.23	1.31	1.41
	R.Q.	0.88	0.82	0.77	0.73	0.75	0.77

GREEN, LIPMANN, OCHOA and LYNEN have recognized in their biochemical studies in vitro on fatty acid oxidation that flavin is a essential component of hydrogen carrying system in this metabolic process. Furthermore, TSUKADA, OSA, NISHINO, TAKEDA and HASHINO in our laboratory have reported that the intravenous administration of the fat emulsion was made effective by the simultaneous administration of riboflavin. Riboflavin is biochemically active only in the form of F. A. D. or F. M. N. (Flavin Mononucleotide). However, in vivo the major part of riboflavin is present in the form of F.A.D., while F.M.N. is found in small quantities and free riboflavin is present in least amount. Therefore, in the present investigation, pure F. A. D. was used. HIKASA and ISHIGAMI observed that simultaneous infusion of ascorbic acid with the fat emulsion improved metabolism of the infused fat, owing to be intensified activity of lipase in serum and the liver. It has also been demonstrated by SUDA and his co-worker's studies that ascorbic acid activates both aconitase and succinic dehydrogenase in the T. C. A. cycle. In fatty acid oxidation, as mentioned above, fatty acid is converted to acetyl coenzyme A by the fatty acid cycle (after Lynen) and, enters into the T.C.A. cycle by condensation with oxaloacetic acid. Accordingly, it is accepted that pantothenic acid, which is a chemical component of coenzyme A, is very important in fat metabolism. Therefore, it is presumed that the relative increase in the R.Q. of liver is caused by an increment of the evolution of carbon dioxide and a decrement of the ketone body production

in this tissue.

The decrease in the R.Q. of the extrahepatic tissues may be caused by the fact that the disposal of ketone body, which is produced in the liver, is markedly reduced, and the direct oxidation of fatty acid, mentioned above, plays an important role in fat metabolism in these cells.

In this group of experiments our results indicate that the infused fat is oxidized more smoothly and completely in all tissues in the case of the simultaneous infusion of methionine and various vitamins with the sesame oil emulsion than in the case of the infusion of the sesame oil emulsion alone or with methionine.

4) Infusion of Cod Liver Oil Emulsion Alone (Group D).

In our laboratory, several kinds of fat emulsions containing triglycerides of various fatty acids were prepared. It is presumed that the metabolic process of fat varies with quality of the fatty acids contained in these emulsions. According to the paper chromatographical studies of Tan, the cod liver oil emulsion contains many highly unsaturated fatty acids, which are not found in the sesame oil emulsion. The author attempted a comparative study of the oxygen consumption and the R. Q. of various tissues in the case of the intravenous administration of these two emulsions. The standard dose of the cod liver oil emulsion was infused intravenously, and the same experiments were repeated. The results obtained are shown in Table 4.

In this case, the rate of increase in Qo_2 of all tissues was slightly lower and

Table 4 Qo_2, Qco_2 and R.Q. of Various Tissues Following Intravenous Infusion of Cod Liver Oil Emulsion into Rats. (Mean)

Time after infusion		0	1 hr.	3 hrs.	6 hrs.	9 hrs.	12 hrs.
Liver	Qo_2	7.30	9.49	13.51	16.06	17.23	18.10
	Qco_2	6.35	6.64	8.51	7.07	9.48	12.31
	R.Q.	0.87	0.70	0.63	0.44	0.55	0.68
Kidney	Qo_2	21.09	21.60	23.85	25.40	28.80	31.68
	Qeo_2	18.35	17.87	18.51	19.30	21.91	25.62
	R.Q.	0.87	0.83	0.78	0.76	0.76	0.81
Spleen	Qo_2	10.15	11.16	12.60	13.39	14.98	16.27
	Qco_2	8.83	9.39	10.22	10.42	11.53	13.50
	R.Q.	0.87	0.84	0.81	0.78	0.77	0.83
Lung	Qo_2	10.51	10.80	11.52	12.10	14.88	15.60
	Qco_2	9.27	9.05	9.24	9.34	11.33	13.12
	R.Q.	0.88	0.84	0.80	0.77	0.76	0.84
Cardiac muscle	Qo_2	5.42	5.76	6.14	6.34	7.08	7.20
	Qco_2	4.72	4.89	4.88	4.92	5.55	6.10
	R.Q.	0.87	0.85	0.79	0.78	0.76	0.85
Skeletal muscle	Qo_2	1.30	1.32	1.35	1.42	1.50	1.58
	Qco_2	1.14	1.14	1.09	1.15	1.13	1.24
	R.Q.	0.88	0.86	0.81	0.81	0.75	0.79

the degree of decrease in the R. Q. of liver was more remarkable than the case of the administration of the sesame oil emulsion. However, the R.Q. of the extrahepatic tissues showed the highest value in all groups. The decrease in the R. Q. of spleen, lung, cardiac muscle and skeletal muscle reached the climax 9 hours after injection of the cod liver oil emulsion, relatively later than in the case of injection of the sesame oil emulsion.

Previously, ASADA and IZUKURA in our laboratory recognized by histochemical studies the fact that the phospholipides, from the infused glycerides, appeared in the hepatic parenchymatous cells in far greater quantities in the case of the administration of the cod liver oil emulsion, which contained many highly unsaturated fatty acids, than the case of the administration of the sesame oil emulsion, not containing them.

Accordingly, a rapid decrease in the R. Q. of liver following the intravenous administration of the cod liver oil emulsion is reasonably explained by the fact that the large amount of phospholipides in this tissue converts chiefly into ketone body by successive β-oxidation. And it is thought that a slighter and slower decrease in the R.Q. of the extrahepatic tissues in this group, as compared with the findings of other groups, was caused by a vigorous disposal of ketone body, which was produced in the liver.

In summary, there was a significant difference between fluctuations in the R. Q. of liver and the extrahepatic tissues following the infusion of the fat emulsion, as shown in Figs. 2, 3, 4, 5, 6, and 7. That is, the decrease in the R. Q. of liver was a far more remarkable than in other tissues, and was most distinct in Group D, next in Group B, then Group A and Group C in order, although this order is just the reverse in the extrahepatic tissues. From these findings it is accepted that liver and the extrahepatic tissues perform respectively different functions in the process of fat metabolism.

B) *CHANGES IN THE RATE OF KETONE BODY PRODUCTION IN VARIOUS TISSUES FOLLOWING THE INTRAVENOUS ADMINISTRATION OF THE FAT EMULSION.*

Fig. 2 Changes in R.Q. of Rat Liver Following Intravenous Infusion of Fat Emulsion.

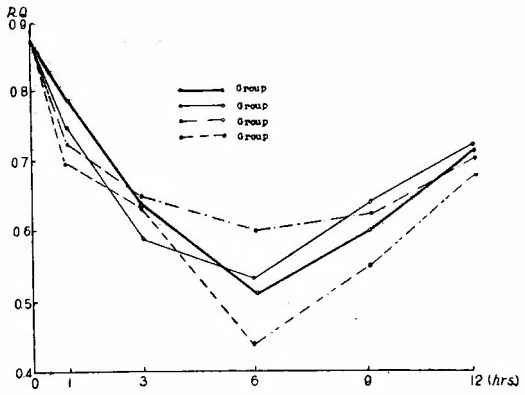


Fig. 3 Changes in R.Q. of Rat Kidney Following Intravenous Infusion of Fat Emulsion.

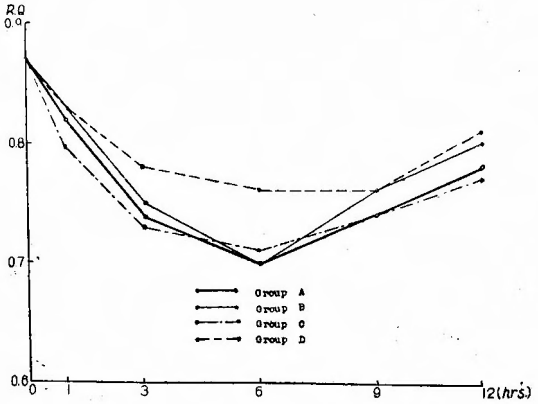


Fig. 4 Changes in R.Q. of Rat Spleen Following Intravenous Infusion of Fat Emulsion.

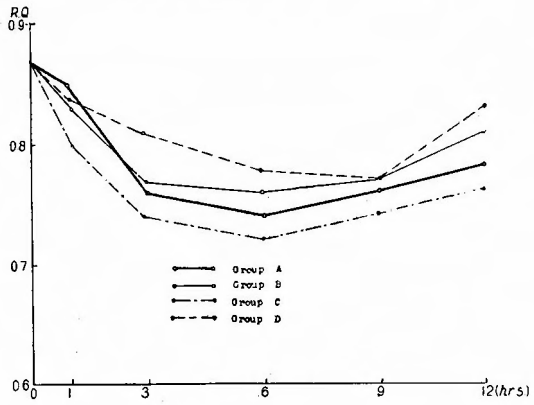


Fig. 5 Changes in R.Q. of Rat Lung Following Intravenous Infusion of Fat Emulsion.

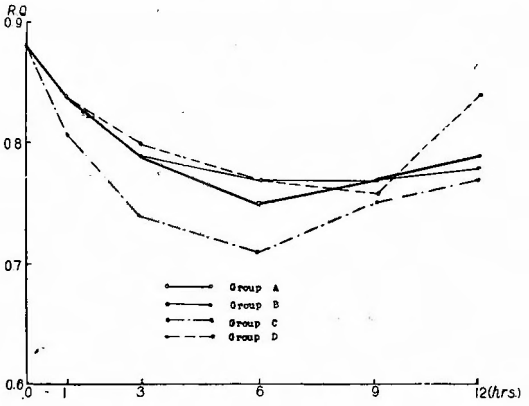


Fig. 6 Changes in R.Q. of Rat Cardiac Muscle Following Intravenous Infusion of Fat Emulsion.

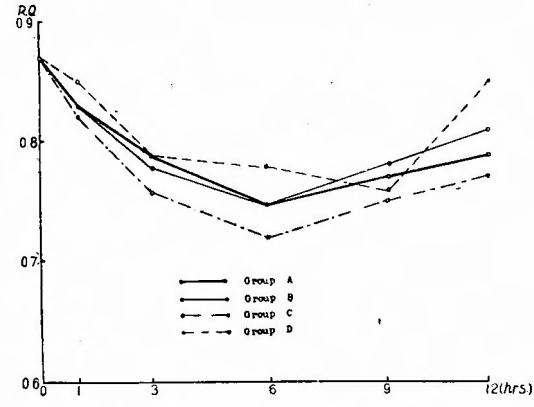
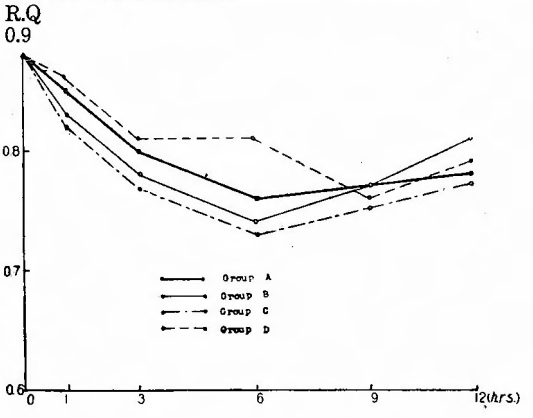


Fig. 7 Changes in R.Q. of Rat Skeletal Muscle Following Intravenous Infusion of Fat Emulsion.



As mentioned above, manometric methods of study on a process of tissue metabolism have a weak point in that the metabolism is observed from the viewpoint of only oxygen and carbon dioxide. For instance, it is possible that the same R.Q. is obtained by different metabolic pathways and a similar value is detected as a total value in various types of metabolism. The exact cause of our decreases in R. Q. is not clearly understood at present.

Therefore, the author carried out determinations of ketone body as acetoacetic acid which was produced in liver, kidney and skeletal muscle following the intravenous administration of fat emulsions.

1) Infusion of Sesame Oil Emulsion Alone.

After the determination on the R.Q. of tissues receiving the standard dose of the sesame oil emulsion, the concentration of ketone body in the medium was measured by the method mentioned above. The rate of ketone body production was expressed as micromoles of acetoacetic acid produced per hour per 100mg of dry tissue (Quacac). The data are presented in Table 5. The Quacac of liver began

Table 5 Rate of Ketone Body Production in Tissues Following Intravenous Infusion of Sesame Oil Emulsion into Rats.
(Values were expressed as μ moles of acetoacetic acid per 100mg dry weight of tissue per hour.)

Time after infusion		0	1 hr.	3 hrs.	6 hrs.	9 hrs.	12 hrs.
Liver	Mean	3.86	4.16	5.57	7.43	5.98	5.29
	Change (%)	0	+ 8	+44	+92	+55	+37
Kidney	Mean	0.81	0.86	0.85	0.89	0.83	0.85
	Change (%)	0	+ 6	+ 5	+10	+ 2	+ 5
Skeletal muscle	Mean	0.66	0.72	0.75	0.73	0.69	0.70
	Change (%)	0	+ 9	+14	+11	+ 5	+ 6

to increase 1 hour after infusion and reached its maximum 6 hours after infusion. Thereafter the rate gradually decreased until it reached the 12 hour stage. On the other hand, the Quacac of kidney and skeletal muscle, which was far lower than that of liver, was hardly increased by the infusion of the sesame oil emulsion.

These results, may be reasonably explained by postulating that a decrease in the R.Q. of liver was caused by an increase in the production of ketone body. However, it may also be that in the extrahepatic tissues the infused glyceride is not only decomposed at the stage of the ketone body, which is produced in liver, but also directly broken down to water and carbon dioxide.

2) Simultaneous Infusion of Methionine with the Sesame Oil Emulsion.

The increase in the Quacac of liver following the simultaneous infusion of methionine, 3mg per 100g body weight, with the standard dose of the sesame oil emulsion was slightly more than that in the case of the sesame oil emulsion alone, as shown in Table 6. However, in kidney and skeletal muscle an obvious increase

Table 6 Rate of Ketone Body Production in Tissues Following Simultaneous Infusion of Methionine with Sesame Oil Emulsion into Rats.
(Values were expressed as μ moles of acetoacetic acid per 100 mg dry weight of tissue per hour.)

Time after infusion		0	1 hr.	3 hrs.	6 hrs.	9 hrs.	12 hrs.
Liver	Mean	3.86	4.32	5.89	7.63	6.01	5.50
	Change (%)	0	+12	+53	+98	+56	+42
Kidney	Mean	0.81	0.82	0.86	0.88	0.85	0.84
	Change (%)	0	+ 1	+ 6	+ 9	+ 5	+ 4
Skeletal muscle	Mean	0.66	0.69	0.72	0.77	0.73	0.70
	Change (%)	0	+ 5	+ 9	+17	+11	+ 6

in ketone body production was not produced by the addition of methionine. These results indicate that methionine accelerates the conversion to ketone body from the infused glyceride in liver, as do the findings obtained by the determination of the

R.Q.

3) Infusion of Cod Liver Oil Emulsion Alone.

In this investigation, instead of the sesame oil emulsion, an equivalent dose of the cod liver oil emulsion was administered intravenously. The results are shown in Table 7. The Quacac of liver increased more markedly when compared with the

Table 7 Rate of Ketone Body Production in Tissues Following Intravenous Infusion of Cod Liver Oil Emulsion into Rats. (Values were expressed as μ moles of acetoacetic acid per 100 mg dry weight of tissue per hour.)

Time after infusion		0	1 hr.	3 hrs	6 hrs.	9 hrs.	12 hrs.
Liver	Mean	3.86	4.11	5.63	8.91	6.74	5.82
	Change (%)	0	+ 6	+46	+131	+75	+51
Kidney	Mean	0.81	0.78	0.85	0.87	0.76	0.79
	Change (%)	0	− 4	+ 5	+ 7	− 6	− 2
Skeletal muscle	Mean	0.66	0.68	0.63	0.68	0.64	0.67
	Change (%)	0	+ 3	− 5	+ 3	− 3	+ 2

results of infusion of the sesame oil emulsion, differing from that of kidney and skeletal muscle.

This fact shows that the infused cod liver oil emulsion, containing many highly unsaturated fatty acids, is chiefly converted into ketone body in liver, while the infused sesame oil emulsion, containing higher fatty acids other than highly unsaturated fatty acids, enters not only into liver, but also directly into the extrahepatic tissues to be oxidized.

4) Simultaneous Infusion of Methionine with the Cod Liver Oil Emulsion.

The Quacac of liver following the simultaneous administration of methionine, 3mg per 100g weight, and the standard dose of the cod liver oil emulsion showed the highest value as compared with that in the above three groups. However, in kidney and skeletal muscle the production of ketone body was hardly increased after

Table 8 Rate of Ketone Body Production in Tissues Following Simultaneous Infusion of Methionine with Cod Liver Oil Emulsion into Rats. (Values were expressed as μ moles of acetoacetic acid per 100 mg dry weight of tissue per hour.)

Time after infusion		0	1 hr.	3 hrs.	6 hrs.	9 hrs.	12 hrs.
Liver	Mean	3.86	4.30	6.65	8.92	7.41	6.04
	Change (%)	0	+11	+72	+131	+92	+56
Kidney	Mean	0.81	0.83	0.89	0.84	0.86	0.87
	Change (%)	0	+ 2	+10	+ 4	+ 6	+ 7
Skeletal muscle	Mean	0.66	0.67	0.69	0.60	0.69	0.67
	Change (%)	0	+ 2	+ 5	− 9	+ 5	+ 2

infusion of the cod liver oil emulsion (Table 8). In short, an increase in ketone body production of liver was most remarkable in the case of the simultaneous infusion of methionine with the cod liver oil emulsion, next in the case of the cod liver oil emulsion, than methionine with the sesame oil emulsion, the sesame oil emulsion alone, in order (Fig 8), although an increase in that of kidney and skeletal muscle

Fig. 8 Changes in Rate of Ketone Body Production in Liver Following Intravenous Infusion of Fat Emulsion.

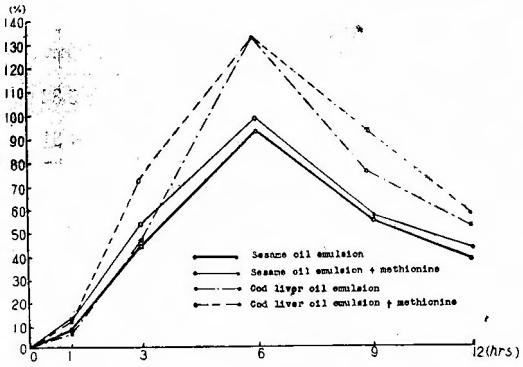


Fig. 9 Changes in Rate of Ketone Body Production in Kidney Following Intravenous Infusion of Fat Emulsion.

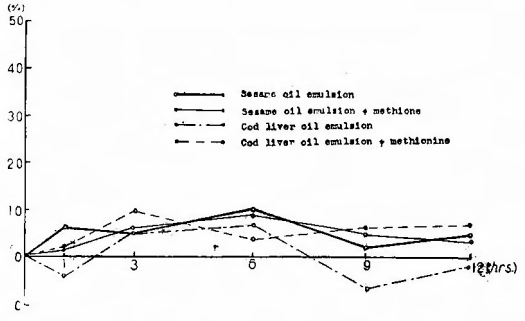
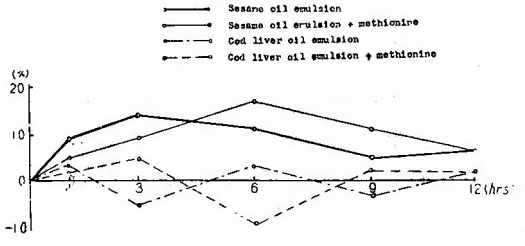


Fig 10 Chages in Rate of Ketoe Body Production in Skeletal Muscle Following Intravenous Infusion of Fat Emulsion.



was not noticed in all cases (Figs. 9 and 10). From these findings, it is evident that ketone body in liver was produced in greater quantity by the infused cod liver oil emulsion than the sesame oil emulsion and the ketone body formation was accelerated by the administration of methionine.

Therefore, we can not help considering that the above mentioned distinct decrease in the R.Q. of liver was caused by the production of ketone body.

CONCLUSIONS

Since the fat emulsion, which could be safely given intravenously, was prepared in our laboratory, Asada, Izukura, Shirotani, Nakata, Seno, Hashino and others have carried out histochemical and biochemical studies on the metabolic process of the infused fat. According to their results, the infused fat globules are first phagocytized by the alveolar phagocytes, stellate cells and reticuloendothelial cells of the spleen from the blood stream, and then glycerides were changed gradually into phospholipides in these cells.

Afterwards, the phospholipides, formed from the infused glycerides entered not only into the hepatic parenchymatous cells, but also into the extrahepatic tissues to be oxidized. On the other hand, ketone body levels in the blood began to increase after infusion of the fat emulsion. In which organ these ketone bodies were produced?

The auther demonstrated in the present paper that the liver is the chief site for the formation of ketone body. However, it is supposed that the metabolic process of fat varies with the kind of fatty acids contained in the administered fat, as has been stated by LEHNINGER, GOLDMAN, CHAIKOFF and others.

The rate of ketone body production in liver following the intravenous administration of the cod liver oil emulsion, containing many highly unsaturated fatty acids showed higher values in comparison with sesame oil emulsion, which does not contain them. The ketone body formation in the extrahepatic tissues even with the addition of methionine was not determined, since ketone bodies, which were produced in these tissues, were immediately broken down to the final stage of fat metabolism. In liver, the major part of fatty acids were converted to the stage of ketone body, which diffused into the blood stream to be finally oxidized in the extrahepatic tissues, while a part of them directly were converted to water and carbon dioxide. Accordingly, the process of fatty acid oxidation divides into direct oxidation, by which fatty acids are directly oxidized in tissues, and indirect oxidation, by which they are oxidized finally in the extrahepatic tissues after conversion to ketone bodies. Furthermore, it is well demonstrated by the results in the R.Q. of tissue slices that various vitamins, like F.A.D., vitamin C and pantothenic acid, play the important role in the catabolic process of fat metabolism, especially in the stage of the T.C.A. cycle.

SUMMARY

The author measured the R.Q. and the rate of ketone body production in rat tissues following the intravenous administration of the fat emulsion produced in our laboratory and obtained the following results:

1) The R.Q. of liver, kidney, spleen, lung, cardiac muscle and skeletal muscle began to decrease 1 hour after infusion of the fat emulsion, reached its minimum titer 6 hours thereafter and then gradually increased.

2) The decrease in the R.Q. of liver was far more remarkable than that in other tissues.

3) The rate of ketone body production in liver increased greatly after infusion of the fat emulsion, while there was no evidence that ketone bodies were produced in the extrahepatic tissues.

4) Methionine enhanced conversion to ketone bodies from the infused glycerides in liver.

5) The infused cod liver oil emulsion, containing many highly unsaturated fatty acids, was chiefly converted into ketone body in liver, however, the infused sesame oil emulsion, containing higher fatty acids other than highly unsaturated fatty acids, entered not only into liver, but also directly into the extrahepatic tissues to be oxidized.

6) The catabolic process of fat metabolism in vivo carried out smoothly and completely by the simultaneous administration of methionine and various vitamins, like F.A.D., vitamin C and pantothenic acid with the sesame oil emulsion.

The author wishes to thank Dr. YORINORI HIKASA for his many valuable suggestions and kind guidance throughout the present investigation.

The present investigation was supported in part py a Research Grant from the Department of Education Science Research Foundation.

REFERENCES

1) Artom, C. & Cornatzer, W. E.: The action of choline and fat on lipide phosphorylation in the liver. J. Biol. Chem., **171**, 779, 1947. 2) Artom, C.: Mechanism of action of lipotropic factors in animals. Liver injury (Transaction of the 10th conference, May, 21-22, 1951, N.Y.), P. 62. 3) Artom, C.: Role of choline in the oxidation of fatty acids by liver. J. Biol. Chem., **205**, 101, 1953. 4) Asada, S.: Histochemical studies on the intravenously infused fat emulsion. Arch. Jap. Chir., **22**, 77, 217, 1954. 5) Baldwin, E.: Dynamic aspect of biochemistry. 1953. 6) Bondy, P.K. & Wilhelmi, A.E.: Effect of hormones upon the production of ketone bodies by rat liver slices. J. Biol. Chem., **186**, 245, 1950. 7) Chaikoff, I. L., Goldman, D. S., Brown, G.W., Dauben, W.Q. & Gee, M.: Acetoacetic formation in liver, I. From palmitic acid1-C^{14}, 5-C^{14} and 11-C^{14}. J, Biol. Chem., **190**, 229, 1951. 8) Drysdale, G. R. & Lardy, H. A.: Fatty acid oxidation by a soluble enzyme system from mitochondria. J. Biol. Chem., **202**, 119, 1953. 9) Fujita A.: Manometric method and its application in the field of biology and medicine. Iwanami, 1949. 10) Geyer, R. P., Chipman, J & Stare, F. J.: Oxidation in vivo of emulsified radioactive trilaurin administered intravenously. J. Biol. Chem., **176**, 1469, 1948. 11) Geyer, R.P., Matthews, L.W. & Stare, F.J.: Metabolism of emulsified trilaurin and octanoic acid by rat tissue slicess. J. Biol. Chem., **180**, 1037, 1949. 12) Geyer, R.P. & Mary Conningham: Metabolism of fatty acids in vitro, studied with odd and even numbers of the RC^{14} OOH series. J. Biol., Chem., **184**, 641, 1959. 13) Goldman, D. S., Chaikoff, I.L., Reinhardt, W. O., Entenman, C. & Dauben, W.G.: The oxidation of palmitic acid-C^{14} by extrahepatic tissues of the dog. J. Biol. Chem., **184**, 719, 1950. 14) Gorens, S.W., Geyer, R.P., Matthews, L. W. & Stare, F. J.: Parenteral nutrition, X. Observation on the use of a fat emulsion for intravenous nutrition in man. J. Lab. and Clin. Med., **34**, 1627, 1949. 15) Grafflin, A.L. & Green, D. E.: Studies on the cyclophorase system. Ⅱ. The complete oxidation of fatty acids. J. Biol. Chem., **176**, 95, 1948. 16) Green, D.E., Goldman, D.S., Mii, S. & Beinert, H.: The acetoacetate activation and cleavage enzyme system. J. Biol. Chem., **202**, 137, 1953. 17) Hashino, H.: Experimental studies of fat metabolism from the viewpoint of ketone body formation. Arch. Jap. Chir., **24**, 488, 1955. 18) Hikasa, Y., Ishigami, K., Asada, S., Zaitsu, A., Tsukada, A. & Nakata, K.: Studies on the intravenous administration of fat emulsion. J.J.S.S., **52**, 298, 1951. 19) Hikasa, Y., Asada, S., Zaitsu, A., Tsukada, A. & Nakata, K.: Studies on the intravenous administration of fat emulsion. Arch. Jap. Chir., **21**, 1, 1952. 20) Hikasa, Y.: Studies on the intravenous administration of fat emulsion prepared by high pressure jet. Rev. Phys. Chem. Jap., **12**, 83, 1952. 21) Hikasa, Y., Asada, S., Zaitsu, A., Tsukada, A. & Nakata, K.: Studies on the intravenous administration of fat emulsion. Arch. Jap. Chir., **21**, 1, 1952. 22) Hikasa, Y, Hasino, H., Osa, H. & Takeda, S.: The role of water-soluble vitamins in fat metabolism. Nipponrinsho, **13**, 1225. 1955. 23) Ikeda, H.: Experimental studies on fat metabolism with a blocked reticuloendothelial system. Arch. Jap. Chir., **26**, 355, 1957. 24) Izukura, T.: Histochemical studies on intravenously adminstered fat emulsion. Arch. Jap. Chir., **26**, 215, 1957. 25) Jowett, M. & Quastel, J.H.: Studies in fat metabolism, I. The oxidation of butyric, crotonic and β-hydroxybutyric acid in the presence of guinea pig liver slices. Bioche. J., **29**, 2143, 1935. 26) Jowett, M. & Quastel, J. H.: Studies in fat metabolism, II. The oxidation of normal saturated fatty acids in the presence of liver slices. Biochem. J., **29**, 2159, 1935. 27) Jowett, M. & Quastel, J.H.: Studies in fat metabolism, III. The formation and breakdown of acetoacetic acid in animal tissues. Biochem. J., **29**, 2181, 1935. 28) Kennedy, E. P. & Lehninger, A.L.: The products of oxidation of fatty acids by isolated rat liver mitochondria. J. Biol. Chem., **185**, 275, 1950. 29) Kuyama, T.: Clinical studies on the nutritional effects of intravenous administration of fat emulsion. unpublished. 30) Lehninger, A.L.: Fatty acid oxidation and the Krebs tricarboxylic acid cycle. J. Biol. Chem., **161**, 413, 1945. 31) Lehninger, A.L.: A quantitive study of the products of fatty acid oxidation in liver suspensions.

J. Biol. Chem., **164**, 291, 1946. 32) Lehninger, A.L.: The oxidation of higher fatty acids in heart muscle suspensions. J. Biol. Chem., **165**, 131, 1946. 33) Leloir, L.F. & Munoz, J.: Fatty acid oxidation in liver. Biochem. J., **33**, 739, 1939. 34) Leloir, L.F. & Munoz, J. M.: Butyrate oxidation by enzymes. J. Biol. Chem., **153**, 53, 1944. 35) Lipmann, F.: On chemistry and function of coenzyme A. Bact. Rev., **17**, 1, 1953. 36) Lynen, F., Wessely, L., Wieland, O. & Rueff, L.: Zur β-Oxydation der Fettssaure. Angew. Chem., **64**, 687, 1952. 37) Lynen, F.,: Functional group of coenzyme A and its metaboic relations, especially in the fatty acid cycle. Fed. Proc., **12**, 683, 1953. 38) Mahler, H.R.: Role of coenzyme A in fatty acid metabolism. Fed. Proc., **12**, 694, 1953. 39) Mann, G.V., Geyer, R.P., Watkin, D.M. & Stare, F.J.: Parenteral nutrition, IX. Fat emulsion for intravenous nutrition in man. J. Lab. and Clin. Med., **34**, 699, 1949. 40) Mc Kibbin, J. M., Pope, A., Thayer, S., Ferry, R. M. & Stare, F. J.: Studies on fat emulsion for intravenous alimentation. J. Lab. and Clin. Med., 488, 1945. 41) Munoz, J. M. & Leloir, L.F.: Fatty acid oxidation by liver enzymes. J. Biol. Chem., **147**, 355, 1943. 42) Nakata, K.: Experimental studies on fat metabolism in the lung. Arch. Jap. Chir., **23**, 445, 1954. 43) Nishino, T.: Laboratory studies on the intravenous administration of the fat emulsion in the light of tissue metabolism. Arch. Jap. Chir., **23**, 607, 1954. 44) Novelli, G.D., Keplan, N.O. & Lipmann, F.: The liberation of pantothenic acid from co-enzyme A.J. Biol. Chem., **177**, 97, 1949. 45) Ochoa, S.: Biosynthesis of tricarboxylic acid by carbon dioxide fixation, I. The preparation and properties of oxalosuccinic acid. J. Biol. Chem., **174**, 115, 1948. 46) Ochoa, &., Weisz-Tabori, E.: Biosynthesis of tricarboxylic acid by carbon dioxide fixation, Ⅱ. Oxalosuccinic carboxylase. J. Biol. Chem., **174**, 123, 1948. 47) Oji, K. & Wada, M.; Fat metabolism in the liver. Sogorinsho, **4**, 1050, 1955. 48) Osa, H:. Experimental studies on the intravenous administration of a fat emulsion for nutritional purpose. Arch. Jap. Chir., **25**, 154, 1956. 49) Otani, S.: On mechanism of adverse reaction caused by intravenous administration of fat emulsion. Arch. Jap. Chir., **25**, 172, 1956. 50) Quastel, J. H. & Wheatley, A. H. M.: Oxidation of fatty acids in the liver. Biochem. J., **27**, 1753, 1933. 51) Quastel, J.H. & Wheatley, A.H.M.: Studies in fat metabolism, Ⅳ. Acetoacetic acid breakdown in the kidney. Biochem. J., **29**; 2773, 1935. 52) Sekine, T., Sasagawa, Y., Morita, S. & Takahashi, H.: Warburg's apparatus, Ⅱ. Nankodo, 1955. 53) Seno, A.: A study of the fat metabolism in the isolated perfused liver. Arch. Jap. Chir. **24**, 179, 1955. 54) Shafiroff, B. G. P., & Frank, C.: A homogenous emulsion of fat, protein and glucose for intravenous administration. Science, **106**, 474, 1947. 55) Shafiroff, B.G.P., Hulholland, J.H. & Roth, E.: Intravenous infusion of concentrated combined fat emulsion into human subjects. Proc. Soc. Exp. Biol. and Med., **72**, 543, 1949. 56) Shafiroff, B. G. P. & Hulholland, J. H.: Effects on human subjects of high caloric potency. Ann. Surg., **133**, 145, 1951. 57) Shafiroff, B.G.P. & Hulholland, J.H.: Effects of intravenous infusious of experimental "instant" fat emulsion into volunteer subjects. Proc. Soc. Exp. Biol. and Med., **91**, 111, 1956. 58) Shirotani, H.: Histochemical studies on fat metabolism by intravenous administration of fatty chyle. Arch. Jap. Chir., **26**, 38, 1957. 59) Stern. J.R. & Ochoa, S.: Enzymatic synthesis of citric acid by condensation of acetate and oxaloacetate. J. Biol. Chem., **179**, 491, 1949. 60) Suda, M. & Hara, M.: Sump. Enzym. Chem., **9**, 66, 1954. 61) Suda, M. & Tanaka, T.: Recent Enzymological Chemistry, **16**, 1955. 62) Takeda, S.: Experimental studies on the effect of riboflavin following the intravenous administration of fat emulsion. Arch. Jap. Chir., **25**, 221, 1956. 63) Tan, N.: unpublished. 64) Tatsumi, W.: Klinische Beobachetungen über die Intravenöse Infusion des Fettes. Arch. Jap. Chir., **26**, 1, 1957. 65) Tsukada, A.: Studies on the intravenous administration of the fat emulsion in the light of protein metabolism. Arch. Jap. Chir., **23**, 215, 1954, 66) Umbreit, W.W., Burris, R.H. & Stauffer, J.; Manometric techniques and tissue metabolism. 1949. 67) Warburg, O.: Biochem. Z. **52**, 51, 1924. 68) Weinhouse, S., Millington, R. H. & Volk, M. E.: Oxidation of isotopic palmitic acid in animal tissues. J. Biol. Chem., 185, 191, 1950. 69) Weinmann, E. O, Chaikoff, I.L., Dauben, W.G., Mildred, G.& Entenman, C.; Relative rates of conversion of the various carbon atoms of palmitic acid to carbon dioxide by the intact rat. J. Biol. Chem., **184**, 735, 1950. 70) Yasuda, M.: Fat metablism. Nisshinigaku, **32**, 501, 1943. 71) Yasuda, M.: Lipides in tissues. Igakunoayumi, **13**, 201, 1952. 72) Yoshikawa, H., Kokura, Y., Sekine, T., Morita, S. & Takahashi, H.: Warburg's appa-

ratus, I. Nankodo, 1954. 73) Zilversmit, D.B., Chaifoff, I.L. & Entenman, C.: Are phospholipides obligatory participants in fat transport across the intestinal wall? J. Biol. Chem., **172**, 637, 1948.

和文抄録

組織呼吸商並びに組織内ケトン体産生量の面から観た脂質代謝に関する実験的研究

京都大学医学部外科学教室第2講座（指導：青柳安誠教授）

重永正之

教室創製の脂肪乳剤をラッテの静脈内へ注入し，ワールブルグ検圧計を用いて各種組織の呼吸商並びにケトン体産生量を測定し，次のような結果を得た．

1) 脂肪乳剤注入後，何れの組織に於ても呼吸商は一旦下降し，6時間乃至9時間後に最低値をとるが，その後再び徐々に上昇する．

2) 脂肪乳剤注入時に於ける組織呼吸商の低下度は，肝臓に於ては肝外組織に於けるそれに比べて特に著明である．

3) 脂肪乳剤注入による組織内のケトン体産生量の増加も，肝臓に於ては著明にみられるが，肝外組織に於てはこれを認めることが出来ない．

4) 脂肪乳剤にメチオニンを併用すると，肝臓に於けるケトン体産生量が更に増加する．

5) 高度不飽和脂肪酸を多く含む肝油乳剤よりも，高度不飽和脂肪酸を除く高級脂肪酸を多く含むゴム油乳剤を用いた方が，肝臓に於けるケトン体産生量が少くて，肝外組織で直接完全酸化をうける割合が多いから，体内各組織では有効的に利用される．

6) 生体内に於ける脂質の異化的代謝過程は，充分な F. A. D.，ビタミンC及びパントテン酸等の各種ビタミン類が存在して初めて完全且つ円滑に行われ得るものである．